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Study of Intercellular Interaction of Ruminal Microorganisms of Beef Cattle

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ABSTRACT

Research objective is the assessment of toxicity level of ruminal fluid of cattle with use of indicator luminescent strains of bacteria developed on the basis of *E. coli*. According to the research results systemic toxic action of ruminal fluid with use of sensory bacterial strains with constitutive luminescence (*E. coli K12 TG1* with clone *luxCDABE* genes *Photobacterium leiognathi 54D10*) were assessed. During the contact of native ruminal fluid with sensory luminescent bacteria *E. coli* dose-dependent inhibition of luminescence in the first seconds of contact was registered. However, by the second minute of contact quenching was up to 5.20±0.59% in baseline environment and decreased after two-time dilution procedure up to 102.56±4.83% with dilution 1:1024). After 30 min of incubation of mixture level of luminescence in the sample with native ruminal fluid reached the control level and over time it increased to 190.59±22.15% after 60 min of contact. Strain *E. coli* katG::lux, where the cloned promoter is responsible for the synthesis of catalase providing the neutralization of hydrogen peroxide demonstrated luminescence induction of 3.0 relative units at the 60th minute of contact. Study of genotoxic effects based on activation of repair proteins RecA/LexA using strain *E. coli* recA::lux, allowed to register luminescence inducing effects of the bacterial cells in contact with native rumen fluid.

Key words: Ruminal microbial flora, cattle, *E. coli*, bioluminescence

INTRODUCTION

Ruminal fluid is a complex water system comprising a mixture of different substrates having unequal degree of dispersion and split, set of hydrolytic and other enzymes, as well as numerous groups of prokaryotic microorganisms and protists. Under the influence of the latter on vegetable food fatty acids with short carbon skeleton, amino acids and their cleavage products, which are involved in the metabolism of the host organism (Shevelev and Grushkin, 2003) are formed.

In case of excess entry of protein with fodder a large amount of ammonia is formed, it inhibits the reaction in cycle of tricarboxylic acids, linking alpha-Ketoglutaric acid and thereby, delaying the use of acetyl-CoA but it also entails ketosis of high yielding cows (Aliyev, 1997). A large amount of ammonia increases pH of ruminal contents, emergence of alkalosis of rumen and disruption of ruminal microbial flora (Aliyev, 1997). Toxic action of phorbol esters on population of ruminal microorganisms is also well-known (Oskoueian *et al*., 2014). However, the question about the toxicity level of components of ruminal fluid and character of their influence on ruminal microbial

flora is still left open (Castillejos *et al*., 2007). Existing complex methods of biotoxicity with use of living organisms (Gitelzon *et al*., 1984) have series of drawbacks consisting in increased labour required, low repeatability of registered data, duration of testing and difficulty in quantitative assessment of analyzed parameters (Maguire, 2014; Badr, 2014).

Biological tests with luminescent bacteria may solve this problem (Kaiser and Esterby, 1991), they often exceed well-known testing systems in rate of action, accuracy, response and relative simplicity, allow simultaneous control of significant number of toxic agents (Mandal *et al*., 2011; Henriques *et al*., 2014; Bu *et al*., 2013; Warawa and Lawrenz, 2014). Laboratory strains of *Escherichia coli* were transformed by methods of genetic engineering and molecular biology using developed recombinant plasmids with *lux*-genes of natural microorganisms, which allowed to obtain sensory bacterial strains with a high background level of luminescence, which showed a decrease in luminescence intensity in response to the toxic effects of environmental factors. Further works in this area allowed to receive strains with relatively low background levels of luminescence, which had cloned indicator promoters before luminescence genes that allow to assess the cell response to environmental factors by the degree of luminescence increase (Manukhov *et al*., 2008). It is possible to design such bacterial strains that specifically react to certain toxicants (active oxygen metabolites, mutagens and heavy metals) or directly demonstrate the response of the target cell to adverse environmental factors (oxidative stress, damage to nucleic acids, synthesis of chaperones or membrane components).

In this regard, the purpose of the present research was to evaluate the toxicity of ruminal fluid of the Kazakh white-headed cows and elucidation of the mechanisms of action on the indicator luminescent bacterial strains designed on the basis of *E. coli*.

MATERIALS AND METHODS

Recombinant strain *E. coli*: Recombinant strain *E. coli K12 TG1* with cloned *luxCDABE* genes *Photobacterium leiognathi* 54D10 (Danilov *et al*., 2002), produced as a lyophilized preparation under the trade name "Ecolum" (HBO "Immunotech", Moscow) was used in the study. Immediately before the study, it was reduced from lyophilized state with 10 mL of cooled distilled water, it was kept at 2-4°C for 30 min, after that the bacterial suspension was adjusted to a temperature of 25°C.

A series of reporter microorganisms with inducible expression of *lux*-genes cloned from different promoters were used as other microbial sensors (Table 1) (I.V. Manukhov, Research Institute for Genetics and Selection of Industrial Microorganisms, Russia). As a result of this genetic organization the used reporter constructions were characterized by originally low level of luminescence but had relatively specific response by its induction under influence of the relevant damaging factor (Zavilgelsky *et al*., 2011).

Table 1: Peculiarities of reporter strain promoters linked with *lux*-genes

Promoters	Function of genes	Assessed impact
FabA	3-hydroxydecanoyl-CoA- dehydratase	Membrane damage
IbpA	IbpA Chaperone	Protein damage
SoxS	Synthesis of response regulator to active forms of oxygen	Oxidative stress
KatG	Synthesis of catalase	Oxidative stress
RecA	Regulatory protein, RecA-activator of SOS-response	Damage of DNA
ColD	Colicin D	Damage of DNA

Before the experiment, the abovementioned strains were grown for 18-24 h in LB-agar with 100 μg mL^{-1} of ampicillin-selective factor of hybrid plasmids and after that the resulting biomass was transferred to a fresh LB-broth and additionally were grown to early exponential phase (OD540 = 0.35 pcs.).

Ruminal fluid: The objects of the study was ruminal fluid of beef cattle of the Kazakh white-headed breed used in experiments in native state after coarse filtration or after centrifugation at 1000 g within 15 min using a centrifuge CM-6M (Elmi, Russia). Serial two-fold dilutions were performed in saline in 96-well plate to 1:1024.

Measurement of biological luminescence: The reaction mixture was formed in non-transparent plate wells, with 100 μg of bacterial biosensors and 100 μg of tested ruminal fluid were added, after that the plate was placed in the sample compartment of luminometer LM-01T (Immunotech, Czech Republic) and was measured in kinetic mode using the program «Kilia» for 60 min in the case with the strain sensor and 120 min using the reporter strain. Level bioluminescence of bacterial strains was expressed in terms of bioluminescent index (BLI).

Statistical analysis: The obtained experimental data were processed using program Statistica (2001).

RESULTS AND DISCUSSION

During the first stage general toxic effect of ruminal fluid was assessed using sensory bacterial strains with constitutive luminescence. This effect is detected according to the quenching of luminescence level in the test sample compared with the control samples and is based on direct link of this phenomenon with general metabolism of cell and its viability. When native ruminal fluid contacted luminescent bacteria *E. coli,* dose-dependent inhibition of luminescence at the first seconds of contact was reported. A similar effect was observed when studying the influence of thrombocytic cationic protein on bioluminescence (Karimov *et al*., 2009).

At the second minute of contact quenching amounted to 5.20±0.59% in the case with original media and decreased in two-fold dilutions to 102.56±4.83% with dilution of 1:1024 (Fig. 1). On the one hand, it could be triggered by aggressive components that are part of ruminal fluid that leads to disruption of membrane integrity and cell death. On the other hand, this phenomenon can be caused by the change of intensity of proton potential due to ingress of cells in an media with lower acidity values. A possible consequence of this effect is the absorption of photons by the components of ruminal fluid because of its turbidity and coloring that is perceived as the toxic effect of the individual components as the increase of luminescence exceeding those in the control samples was observed during further study of luminescence kinetics of bacterial cells reference.

So, after 30 min of incubation the luminescence level of the sample with native ruminal fluid reaches the control and with time increases up to 190.59±22.15% 60 min after the contact. Moreover, the dilution of initial liquid under study allowed to register even greater stimulation of luminescence of biosensors at titer 1:16 that reach peak values and is 1223.52±119.58% of the control values. Further dilution up to 1:1024 gives no additional stimulation of luminescence and simultaneously exerts no inhibitory effect in the first minutes of contact. On the other hand, using an extra centrifugation procedure of ruminal fluid at 1000 g within 15 min in order to settle the suspended particulate matters did not result in significant changes of the received results and allowed to recommend the evaluation of a given biological fluid in initial native state.

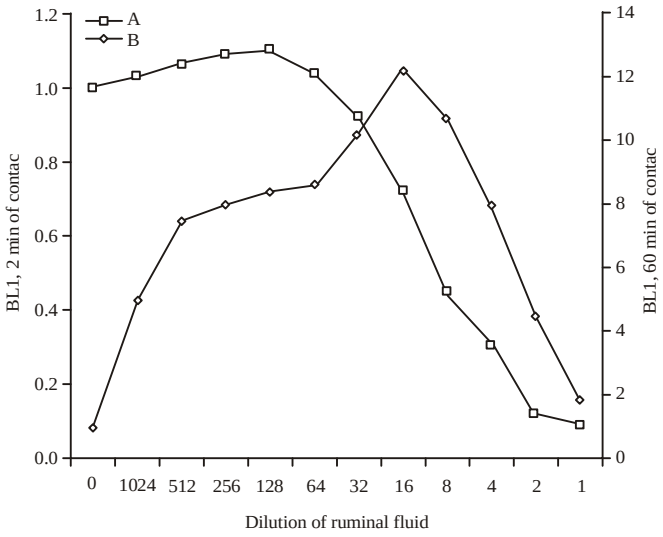

Fig. 1: Level of luminescent response of sensor strains *E. coli* at (A) 2 and (B) 60 min of contact with ruminal fluid having different dilution

The received data on the assessment of the general toxicity characterize ruminal fluid as sufficiently favorable media for bacterial growth, despite initial disintegration effect on bacterial cells. It is provided by an abundance of nutrient substrates of different level of fermentation in this media, including even such easily accessible metabolite as glucose (Aliyev, 1997).

Further studies were aimed to identify the nature of the influence of ruminal fluid on bacterial cells and to determine the peculiarities of cells response. In this case, if in the media there is a factor of activation of specific promoter, integrated directly before *luxCDABE* genes; expression of the latter begins that is reflected by an increase of luminescence.

Use of the strain *E. coli fabA:lux*, where, the used promoter is responsible for the synthesis of 3-hydroxydecanoyl-CoA-dehydratase, which is a key enzyme of biosynthesis reactions of membrane fatty acids and also the strain *E. coli ibpA::lux*, where the specific promoter is responsible for the synthesis of heat shock chaperone binding proteins and facilitating their subsequent refolding by the ATP-sensitive chaperones (Matuszewska *et al*., 2008), allowed to register no significant effects (Fig. 2). However, the strain *E. coli katG::lux*, wherein, the cloned promoter is responsible for the synthesis of catalase providing the neutralization of the hydrogen peroxide demonstrated induction of luminescence reaching 3.0 relative units at the 60th min of contact. This fact attests to the presence of active oxygen forms, primarily, the stable molecules of hydrogen peroxide able to diffuse at large distance in the media and freely penetrate through membranes of cell. Despite the fact that superoxide anion is the precursor of this form of oxygen metabolites, it was not registered in the media, that fact is confirmed by the absence of the response of the strain *E. coli soxS*::*lux*, where the specific promoter is responsible for the synthesis of regulatory protein in oxidative stress system. On the other hand, it does not negate the possibility of the presence of superoxide anion in the media but because of its low life expectancy, it migrates to the small distance from the place of its formation and is accepted by many components of the ruminal fluid.

Study of genotoxic effects based on activation of proteins of repair system RecA/LexA using strain *E. coli recA::lux* allowed to register effects of luminescence inducing of the bacterial cells in contact with native ruminal fluid. Such result is due to either direct effect of hydrogen peroxide on DNA molecule or the formation of hydroxyl radical from peroxide, causing disturbances in the

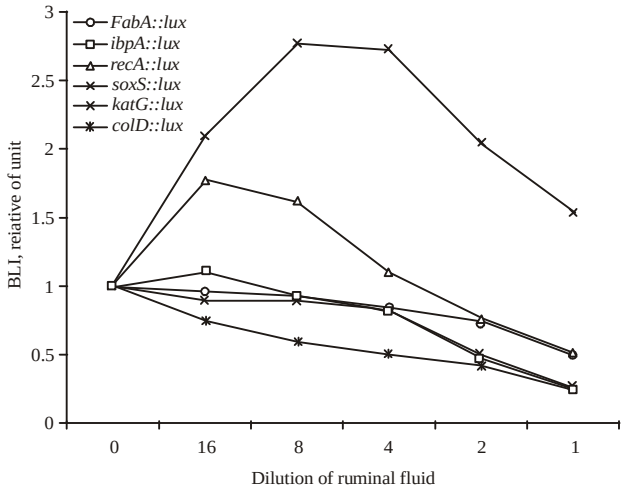

Fig. 2: Luminescence induction of reporter strains at contact with ruminal fluid having different dilution

structure of nucleic acids, provided that the bacterial cell has no defense mechanisms against this metabolite. On the other hand, such genotoxic effects were not observed for the strain *E. coli colD::lux* and the level of luminescence did not increase in contact with ruminal fluid.

CONCLUSION

As a result, the conducted research demonstrated the possibility to assess the toxicity of ruminal fluid using sensor strains of bacteria, which level of luminescence is a direct reflection of disintegrating action of media on cellular components. At the same time, two-stage dependency of changing luminescence was shown; it consisted in its initial quenching with subsequent reviving exceeding control. On the other hand, assessment of differential biological toxicity revealed the formation of oxygen active forms, able to influence on different components of bacterial cell, including DNA that is a variant of genotoxic effect.

REFERENCES

Aliyev, A.A., 1997. Metabolism of Ruminants. MNITS, USA., Pages: 419.

Badr, C.E., 2014. Bioluminescence imaging: Basics and practical limitations. Methods Mol. Biol., 1098: 1-18.

Bu, L., X. Ma, Y. Tu, B. Shen and Z. Cheng, 2013. Optical image-guided cancer therapy. Curr. Pharm. Biotechnol., 14: 723-732.

Castillejos, L., S. Calsamiglia, A. Ferrer and R. Losa, 2007. Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 132: 186-201.

Danilov, V.S., A.P. Zarubina, G.E. Eroshnikov, L.N. Solovyova, F.V. Kartashev and G.B. Zavilgelsky, 2002. Sensor bioluminescent systems on the basis of lux-operons of different types of luminescent bacteria. Biology, 3: 20-24, (In Russian).

Gitelzon, I.I., E.K. Rodicheva and S.E. Medvedeva, 1984. Luminescent Bacteria. Science Press, Novosibirsk, Pages: 275.

Henriques, C., A. Henriques-Pons, M. Meuser-Batista, A.S. Ribeiro and W. de Souza, 2014. *In vivo* imaging of mice infected with bioluminescent Trypanosoma cruzi unveils novel sites of infection. Parasites Vectors, Vol. 7.

Kaiser, K.L. and S.R. Esterby, 1991. Regression and cluster analysis of the acute toxicity of 267 chemicals to six species of biota and the octanol/water partition coefficient. Sci. Total Environ., 109: 499-514.

Karimov, I.F., Y.B. Ivanov and D.G. Deryabin, 2009. Influence of thrombocyte cationic protein on bioluminescence and viability of the recombinant *E. coli* strain with the cloned lux- operon Photobacterium Leiognathi. Herald Orenburg State Univ., 2: 138-142.

Maguire, C.A., 2014. Bioluminescence-based monitoring of virus vector-mediated gene transfer in mice. Methods Mol. Biol., 1098: 197-209.

Mandal, P.K., A.K. Biswas, K. Choi and U.K. Pal, 2011. Methods for rapid detection of foodborne pathogens: An overview. Am. J. Food Technol., 6: 87-102.

Manukhov, I.V., V.Y. Kotova, D.G. Mal'dov, A.V. Il'ichev and A.P. Bel'kov, 2008. Induction of oxidative stress and SOS response in Escherichia coli by vegetable extracts: The role of hydroperoxides and the synergistic effect of simultaneous treatment with cisplatinum. Microbiology, 77: 523-529.

Matuszewska, E., J. Kwiatkowska, D. Kuczynska-Wisnik and E. Laskowska, 2008. Escherichia coli heat-shock proteins IbpA/ B are involved in resistance to oxidative stress induced by copper. Microbiology, 154: 1739-1747.

Oskoueian, E., N. Abdullah, W.Z. Saad, A.R. Omar and Y.W. Ho, 2014. Dose-response Effects of Phorbol Esters Isolated from Jatropha Meal on Rumen Microbial Activities Asian J. Anim. Vet. Adv., 9: 37-46.

Shevelev, N.S. and A.G. Grushkin, 2003. Morphological and functional features of a mucous membrane of rumen of ruminants. Agric. Biol., 6: 15-22.

Statistica, 2001. Data Analysis Software System. Version 6, StatSoft Inc., Tulsa, Oklahoma, USA.

Warawa, J.M. and M.B. Lawrenz, 2014. Bioluminescent imaging of bacteria during mouse infection. Methods Mol. Biol., 1098: 169-181.

Zavilgelsky, G.B., V.Y. Kotova and I.V. Manukhov, 2011. Nanoparticles of titanium dioxide (TiO2) induce stressful reactions fixed by specific lux-biosensors in bacteria. Russian Nanotechnol., 6: 401-405.